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Effects of doses of ascorbic acid on physio-biochemical parameters of Sahel bucks exposed to stocking and 28 –hour road transportation (North Western – South Western, Nigeria)

[1]*Biobaku, K.T., [3]Ameen, S. A., [2]Jibir, M.

[1]*Department of Veterinary Pharmacology and Toxicology, University of Ilorin, Ilorin*
[2]*Department of Animal Science, Usmanu Danfodiyo University, Sokoto.*
[3]*Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ilorin, Nigeria.*

***Corresponding author's e mail:** biobaku.kt@unilorin.edu.ng*

***Target audience:** Farmers, marketers and researchers in the livestock Industry*

Abstract

Sahel bucks were used in evaluating doses of ascorbic acid exposed to long term transportation stress. Their ages ranged between 1.5-2 years, their body condition score was 3 out of 5 scale. Test groups were ascorbic acid at 400mg/kg orally administered; ascorbic acid at 300mg/kg PO; ascorbic acid at 200mg/kg PO and control group without ascorbic acid administration. Thus, 16 animals were used for high stocking rate and 16 stocked using low stocking rate in the experiment. Physiological and biochemical parameters were assessed. The heart rates of the bucks treated with 200mg/kg ascorbic acid midway into the journey were low ($P<0.05$) compared to those treated with 300 and 400mg/kg, and the control group. High dose of ascorbic acid (400mg/kg) produced significantly higher values of sodium and chloride serum concentrations. Mg levels were significantly ($P<0.05$) lowered with lower doses of ascorbic acid treatment compared to the control. In contrast, T_4 values in all treated groups were lower than that of the control animals. Ascorbic acid caused a significant ($P<0.05$) increase in red blood cells; the leucocytes and lymphocytes counts. Conclusively, ascorbic acid at 300 mg/kg and 400 mg/kg had better anti-stress property that could be used to improve animal welfare in bucks exposed to long road transportation.

***Key Words**: Ascorbic acid; Doses; Stress; Sahel bucks.*

Description of Problem

It is important to promote the welfare of animals by attenuation of stress of long road transportation (1). Experience had shown that animals are seen dead along the road side while travelling from the Northern part to the Southern part of Nigeria, Abeokuta. These losses incurred by farmers and marketers amounts to millions of dollars and could affect the agricultural economics. The losses incurred in the livestock industry due to stress long road transportation are not well documented. But Government of Nigeria had created stipulated codes and legislation concerning animal welfare, which protects animals from potentially stressful stimuli that may compromise their welfare. These are laws are not fully enforced in many countries of the world or not enforced appropriately (2).This includes the Sub-Saharan countries including Nigeria (3).

Fear responses in a particular situation are difficult to predict because they depend on how the animals perceived their handling or transport experience (4). The amygdala in the brain is probably the central fear system that is directly involved with fear behaviour and the acquisition of conditioned fear, while the sub-cortical pathway is associated with fear and extinguishing fear condition (5). There is no universal agreement on what constitute stressful stimuli in animals, method of quantifying the animal's response to stressful stimuli and the ways by which stress can be ameliorated (2). The use of supplements in the prevention of stress in food animals, especially in West Africa is uncommon in long term transportation stress, therefore proper evaluation of appropriate doses for amelioration of stress and improvement of welfare animals subjected to long road transportation. Improving the wellbeing and transportation conditions of animals to remove or minimize stressful stimuli is both ethically and economically desirable because it improves the meat quality and minimizes transportation mortality (6). In the same vein, many pharmacological agents used to ameliorate stress have long withdrawal period. Most of them are not adhered to and have public health implications. Unlike ascorbic acid with no withdrawal period and with utmost safety to both transported animals and target consumers. The supplementation of ascorbic acid is said to improve temperature in pigs after at short term transportation of 2 hours in pigs (7). This study is aimed at evaluating effects of ascorbic acid in Sahel bucks exposed to long term stress of over 28 hrs of road transportation. It also try to assess the doses of ascorbic acid doses recommendations adhering to standard regulations for transportation for goats (low stocking) alongside non- standard high (stocking rate) on physiological and biochemical parameters .

Materials and methods

Location of study Area

The experimental journey started livestock farm unit of Faculty of Agriculture, Usmanu Danfodiyo University, Sokoto, Nigeria. (13.1^{o}N and longitude 5^{o}13$^{'}$E 350m above sea level). The goats were transported during the Harmattan season in the month of January. The journeys ended at Federal University of Agriculture, Alabata, Abeokuta, and Ogun State (latitude 71^{o} 0'N, longitude 32$^{o'}$E at an altitude of 76m above sea level). The distance travelled during the experimental journey was about 969km which took 28 hours. The speed maintained was journey was 40km/hr which was in conformity with the Animal Disease Control Act of 1988 of the Federal Republic of Nigeria. This act was in accordance to international laws of transportation of animals (8). The experimental journey previously conducted (9).

Experimental Animals and Management

Thirty-two apparently healthy Sahel bucks served as the population for the experiment. Their ages ranged between one and half to two years, weighed between 10-14 Kg and had their universal body score of 3 out of 5 scale. The animals were purchased from Kware, and Gudu livestock markets, Sokoto State. The animals were acclimatized for two weeks and were clinically examined and treated against gastrointestinal parasites as helminthes using albendazole (Albenzole, Agbara Industries, Lagos, Nigeria) and a combination of penicillin and streptomycin (penstreptomycin® Kepro, Holland) was used against secondary bacterial infections. The bucks were fed on groundnut hay, cowpea husk and corn residue the animals were also served with clean fresh water *ad libitum* ,they were not restrained in the pen and were stocked at a rate above 2m^2/goat as previously research (10). The animal was managed semi-intensively during the two weeks of acclimatization to prevent predisposition to stress.

Experimental Design

A total number of 32 animals were used in the study comprising of 8 groups of four bucks per group, Group I: bucks treated with Ascorbic acid at 400mg/kg orally administered (PO); Group II: were treated with ascorbic acid at 300mg/kg PO; Group III : ascorbic acid at 200mg/kg PO; while Group IV: were the control non-treated group. Each of treatments has low and high stocking rates respectively. The doses were chosen as previously adopted (11). The animals were stocked using a high stocking density which was not in conformity with standard international regulation and a standard low stocking rate which is in line with the Animal Disease Control Act of 1988 of Nigeria and is in agreement with standard international regulations for transportation of goats. Thus, 16 animals were experimented upon for high stocking rate and 16 stocked using low stocking rate.

Table 1: Effects of ascorbic acid and stocking rate on physiologic parameters of Sahel bucks in long term transportation stress

	Dose					Stocking rate			
Parameter	200mg/kg	300mg/kg	400mg/kg	Control	SEM	High	Low	SEM	Interaction
HRp b/min	88.71	85.00	83.00	102.32	2.54	84.16	81.83	4.53	NS
HRm b/min	70.75^{b}	91.63^{a}	93.00^{a}	102.38^{a}	2.91	88.81	95.44	2.063	NS
HRe b/min	53.00^{b}	46.00^{b}	45.75^{b}	104.75^{a}	2.34	68.18	55.31	1.652	*
C/min	35.13	38.88	40.50	35.13	2.27	35.75	40.54	1.85	NS
Rm	34.75^{a}	42.12^{a}	32.38^{b}	25.63^{c}	1.65	31.69	32.75	1.17	*
Re C/min	41.25	40.25	36.42	33.38	1.76	39.39	36.06	0.92	*
Tp ^{o}c	39.98	39.10	39.93	39.67	0.20	39.18	40.15	0.17	NS
Tm ^{o}c	39.15	39.31	39.26	39.31	0.21	39.35	39.15	0.15	*
Te ^{o}c	39.23^{a}	38.85^{a}	38.65^{a}	39.15^{a}	0.19	39.00	38.88	010	NS
ESp	3.88	3.88	4.00	3.88	0.08	4.00	3.83	0.08	NS
ESm	2.75	2.88^{a}	3.75^{b}	4.00^{a}	0.27	3.25	3.38	019	NS
ESe	4.00^{a}	4.00^{a}	4.13^{a}	4.00^{a}	0.06	4.07	400	004	NS

abc means bearing different superscripts along the same row differ by (P<0.05)
While NS means not significant,*Significant (P<0.05) HR: Heart rate; HRp: Heart rate (prior); HRm : Heart rate (midway);Heart rate; HRe Heart rate (end); Rp: Respiratory rate (prior); Rp: Respiratory rate (midway); Rp:Respiratory rate (end); Tp^{o}c:Temperaure (prior),Tm^{o}c:Temperaure (midway); Te^{o}c: Temperaure (end); ESp :Excitatory score (prior); ESm : Excitatory score (midway); ESe : Excitatory score (end)

Table 2: Effect of varying doses of ascorbic acid on serum electrolytes mean* in Sahel bucks exposed to long term transportation

		Ascorbic acid Dose		
Parameters	Control	(200mg/kg)	(300mg/kg)	400mg/kg
ALT(IU /l)	112.53±25.04^{b}	122.64±49.71^{a}	91.07±17.19^{c}	96.54±12.26^{c}
AST (IU /l)	246.39±43.52	232.52±77.28	226.44±73.04	232.07±78.76
Glucose (mg/dl)	82.22±15.48	70.22±14.06	71.55±22.32	77.33±18.51
Cholesterol (mg/dl)	90.52±49.57^{b}	73.10±23.47^{c}	87.33±35.43^{b}	102.43±41.49^{a}
Protein (g/l)	73.60±12.04	72.30±9.95	75.07±5.93	68.52±11.21

Values with different superscripts abc along the same row were significantly different (P<0.05)
ALT: Alanine aminotransferase ; AST: Aspartate aminotransferase
mean*: (Mean ± SD)

Loading, Stocking and Experimental Journey Ethics

A transportation and health certificate was collected to reaffirm the animal's health status from the Ministry of Forestry and Animal Health of Sokoto State. The Sahel bucks were handled with carefully and loaded in to the vehicle. The journey commenced at 09:00 am and animals were loaded at low stocking density (rate) were stocked at $0.30m^2$per animal; while the animal at high stocking density was stocked at $0.15m^2$ per animal as previously conducted (10). The animals transported at low stocking density were in accordance to international guidelines on transportation of animals (1, 8). The animals transported at low stocking density were rested 12 hours into the journey at Jebba town (latitude 90^o.06' and 9^o55' N and longitudes 4^o 02' and 4^o 05'E).Resting the animals was carried out in conformity as stipulated in the animal transportation act Nigeria (8) .This is in concordance with the international guidelines for loading and transportation (9).

Administration of Ascorbic acid

Ascorbic acid oral tablets Spartan®-C (Agbara Drug Company, Lagos, Nigeria) was used in the current study. The doses of administered orally were graded doses deduced from previous research (12) and (13). Ascorbic acid was orally administered in the beginning at Sokoto and at Jebba mid-way in to the experimental journey at following doses 400mg/kg; Group II: 300mg/kg; Group III:200mg/kg; while Group IV: were in the control non-treated group.

Measurement of Vital Physiologic Parameters and Weight

All the vital parameters were taken pre-loading at Sokoto where the experimental journey began; at Jebba midway and after off-loading of the animals at Abeokuta. Rectal temperature was measured using a stethoscope and using a time set using standard procedure as previously described (3). Excitability score was also assessed as in previous studies (12) and was adapted in which the score of 1-4 lower score indicate calmness while higher scores indicates anxiety.

Blood Collection, Haematological and Biochemical Analysis

Blood samples were collected from each animal at preloading, midway into the journey and post loading 12 hours into the journey and when resting the animal's midway at the veterinary quarantine post and after off loading as in a previous research (10). The blood was collected using standard procedure as previously described in a previous research.

Full blood count and packed cell volume (PCV), and erythrocytes indices such Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH),Mean Corpuscular Haemoglobin Concentration (MCHC) were carried out using the method previously described (14).The parameters determined were red blood cell count (RBC) as previously adopted (14).Erythrocytes sedimentation rate and haemoglobin concentration were determined using the standard method (15).Total white blood cell, differential count and the neutrophils–lymphocytes ratio (N:L) were determined as previously adopted in previous researches (14,15, and 16).Randox® commercial test kits (England) were used to determine the biochemical parameters using standard spectrophotometeric procedures. Alanine aminotransferase (ALT).Aspartate amino transferase (AST), glucose was determined using glucometer (Roche Diagnostics Germany) and was used as described by the manufacturers guide as previously conducted (12).Biochemical parameters carried out are cholesterol, and protein. The electrolytes analyses that was carried using commercial test kit (Randox®, England). Antioxidative stress markers assayed were glutathione transferase as adopted in previous research (17). (Superoxide dismutase was also assessed using the method described in a previous research (3); malondaialdehyde enzyme was assessed using thiobarbituric acid reacting substances assessment the method (19) and as previously adopted (3) .Thyroid hormones namely triiodothyronine T3 and tetraidothyronine T4 were also assayed using Biorex® test kit .Biorex® Diagnostics limited United Kingdom) and ELISA was used in previous researches (20 ,21, 22)

Result and Discussion

The effect of varying doses of ascorbic acid and stocking rate on the heart rate, respiratory rate, temperature and excitability scores of bucks prior, midway and post transportation are presented in table 1.The heart rates of the bucks treated with 200mg/kg ascorbic acid midway into the journey were lower compared to those treated with 300 and 400mg/kg, and the control groups ($p<0.05$). The heart rate of the control at the end of the journey was significantly ($P<0.05$) higher than those of the treated groups. The heart rates obtained for ascorbic acid treated groups at the end of the journey were lower than those recorded before and midway into the journey. In this study, ascorbic acid has a non-significant effect on heart rates changes and cardiac cycle. This could be due to the indirect involvement of ascorbic acid in synthesis of catecholamine and neurotransmitters and might be linked with excitability score and mood of the animals, this is in agreement with the findings of previous research (15). Stocking rate did not significantly ($P>0.05$) affect the heart rates of the transported animals before, midway and after the journey, however, the heart rates obtained at the end of the journey were the lowest when compared to those obtained prior and midway into the journey.

The respiratory rates of the animals treated with varying doses of ascorbic acid before, midway and after transportation of the bucks appear not to be significantly different, from the values of midway into the journey, when respiratory rate of the control group was significantly ($p<0.05$) reduced compared to the treated groups. The group treated with 400mg/kg ascorbic acid was significantly ($p<0.05$) lower than those of 200mg/kg and 300mg/kg treated groups.

The temperatures of the bucks given ascorbic acid at various doses were not significantly different from the control, prior, midway and after the journey. These findings are not in line with the findings of previous work (7), this may be due to the fact that the animals used in this study are of different species from the animals used in the previous study. The season in which the study was conducted was in the harmattan while the hot dry season was used in previous study (7).

The excitatory scores were not significantly different in the treated groups and the control except midway into the journey, where the group treated with 200mg/kg had decreased significantly ($P<0.05$) excitatory score when compared to the groups treated with higher doses of ascorbic acid and the control. In a previous study supplementation improved the excitability score in virtually all treatment groups during long term transportation which was in line with a previous study conducted (12,15). There was also good effect on excitability score at slaughter. The ascorbic acid would have been involved indirectly, since it is associated with neurotransmitter synthesis. Inferences could be made that the electrical activities in the brain modulated by ascorbic acid might have influenced the temperament of the animals during journey. The findings in this study was different due to the doses employed, time of induction of transportation of stress in which is longer in this study compared to the previous. The breed of the animals also are different in the previous study the Sokoto red was the animal transported unlike this study the Sahel breed was used as a model and they are more domesticated and observation had shown that they are calmer and hardier.

Table 3: Effect of different doses of ascorbic acid on serum electrolyte mean* in Sahel bucks exposed to long term transportation stress

		Ascorbic acid Doses		
Parameters	**Control**	**(200mg/kg)**	**(300mg/kg)**	**400mg/kg**
Ca (mg/dl)	9.63±0.79	9.31±0.56	9.18±0.47	9.22±0.44
Mg (mg/dl)	2.73±0.468 [a]	2.57±0.49 [b]	2.57±0.67 [b]	2.73±0.568 [a]
Na (mg/dl)	147.83±6.28 [b]	145.06±6.79 [c]	148.93±2.21 [b]	157.04±63.22 [a]
K (mg/dl)	5.28±0.10	6.11±0.21	6.46±0.08	5.87±0.22
Cl (M mol/l)	102.89±1.37 [c]	116.611±1.15 [b]	96.14±1.37 [d]	129.60±1.37 [a]

Values with different superscripts abcd along the same row differ significantly (P<0.05)
mean* : (Mean ± SD)

Table 4: Effect of varying doses ascorbic acid on antioxidative stress markers and thyroid hormones mean* in Sahel bucks in long term transportation stress

		Ascorbic acid Dose		
Parameters	**Control**	**(200mg/kg)**	**(300mg/kg)**	**(400mg/kg)**
GST (μ/ml)	1.12[c]±0.54	1.26[b]±040	1.35[a]±0.29	1.43[a]±0.30
MDA ($M^{-1}cm^{-1}$)	0.05[c]±0.46	0.07[b]±0.06	0.11[a]±0.10	0.10[a]±0.12
SOD (Iμ/L)	33.21±11.24	33.44±15.17	43.65±16.32	40.93±17.22
T4(μg/dl)	9.30[a]±0.10	8.15[b]±0.21	7.22[c]±0.08	7.03[d]±0.22
T3 (nl/ml)	2.81±1.24	2.96±1.23	2.69[c]±1.25	2.73[b]±1.28

Values with different superscripts abc along the same row differ significantly (P<0.05)
GST: Glutathione-S-Transferase
MDA: Malonyldialdehyde
SOD: Superoxide Dismutase
T_3: Triiodotyronine
T_4: Tetraiodotyronine
mean* : (Mean ± SD)

Table 2 shows that there was a significant decrease (P<0.05) in the ALT in all groups except at the low dose (200mg/kg) of ascorbic acid compared to the control. The cholesterol values were significantly (P<0.05) lower in groups treated with doses of 200mg/kg and 300mg/kg, and higher in 400mg/kg treated groups compared with the control.

Significant (P<0.05) changes in electrolytes were also observed in the treatment groups. High dose of ascorbic acid (400mg/kg) produced significantly higher values of sodium and chloride serum concentrations. Mg levels were significantly (P<0.05) lowered with lower doses of ascorbic acid treatment compared to the control.

MDA was significantly higher (P<0.05) in all ascorbic acid treated groups compared to the control. In contrast, T_4 values in all treated groups were lower than that of the control animals. In the same vein, higher doses of ascorbic acid (300 and 400mg/kg) lead to lower levels of T_3 compared to the control.

In this study, the ascorbic acid improved the biochemical parameters and influenced most parameters especially in the groups that were administered with and 300mg/kg and 400mg/kg doses of ascorbic acid. Ascorbic acid possesses cytoprotective property that would have protected the hepatocytes and prevented the damage due to stress .The liver enzyme (the ALT) was seen to be lower in the group that was administered with 300mg/kg and 400mg/kg of ascorbic acid. This indicated that the ascorbic acid prevented degenerative changes in the hepatocytes. The cholesterol was also lower in the group that was administered with higher of doses (300mg/kg and 400mg/kg) of ascorbic acid and this is most probably due to the involvement of fat in the absorption of other endogenously produced vitamins such as vitamin E and K which might be involved in stress. Ascorbic acid is also indirectly involved in the metabolism of vitamin E , the metabolic role of ascorbic acid might be the reason .The other reason is ascorbic acid might be involved metabolism of lipid at gluconeogenesis and other intermediary pathways. Cholesterol could also be involved in the maintenance of the phospholipids membrane of blood cells to prevent haemolysis as a result of phospholipid synthesis.

Table 5: Effect of varying doses ascorbic acid on erythrocytes parameters and indices mean* of Sahel bucks exposed to long term transportation stress

		Ascorbic acid Dose		
Parameters	**Control**	**(200mg/kg)**	**(300mg/kg)**	**400mg/kg**
RBC x (10^6/µl)	9.30±3.19	10.50±2.79	10.94±3.52	10.93±2.52
PCV (%)	27.10±4.55	24.94±3.85	28.77±3.93	26.58±4.54
ESR (mm/hr)	6.81±1.86	7.00±1.19	7.00±1.94	6.68±2.12
Hb (g/dl)	9.04±1.58	8.60±1.39	8.88±1.63	8.95±1.29
MCHC (Hg/dl)	33.70±5.88	33.54±5.06	32.12±5.26	34.11±5.91
MCH (pg)	8.76±2.69	8.5±1.80	8.58±1.97	10.20±3.28
MCV(fl)	26.25±7.45	24.73±4.61	27.47±6.35	28.53±7.87

$P<0.05$ is considered significant
RBC: Red Blood Cell Count
PCV: Packed Cell Volume
ESR: Erythrocytes Sedimentation Rate
Hb: Haemoglobin Concentration
MCHC: Mean Corpuscular Haemoglobin Concentration
MCH: Mean Corpuscular Haemoglobin
mean* : (Mean ± SD)

Table 6: Effect of ascorbic acid different dose on Leucocytes mean* of Sahel bucks exposed to long term transportation stress

		Ascorbic acid Dose		
Parameters	**Control**	**(200mg/kg)**	**(300mg/kg)**	**(400mg/kg)**
WBC × 10^3(μ/l)	$1.09{\pm}3.35^{c}$	$1.10{\pm}3.12^{b}$	$1.20{\pm}3.149^{a}$	$1.20{\pm}3.80^{a}$
Neutrophils (%)	30.35±8.98	33.72±6.63	32.44±4.88	30.38±4.28
Lymphocytes (%)	$63.12{\pm}9.00^{c}$	$63.17{\pm}8.67^{b}$	$66.17{\pm}5.18^{a}$	$66.61{\pm}3.94^{a}$
N:L	$0.52^{d}{\pm}0.10$	$0.56^{c}{\pm}0.16$	$0.51^{b}{\pm}0.48$	$0.51^{a}{\pm}0.46$
Eosinophils (%)	1.53±1.52	1.94±1.58	1.38±1.12	1.77±1.83
Basophils (%)	0.99±0.58	0.83±0.40	0.22±0.1	0.94±0.50
Monocytes (%)	$0.43{\pm}0.86^{c}$	$1.11{\pm}0.64^{a}$	$0.72{\pm}1.00^{b}$	0.00±0.00

Values with different superscripts abc along the same row differ significantly ($P<0.05$)
WBC: White Blood Cell Count
N: L: Neutrophils Lymphocytes Ratio
mean* : (Mean ± SD)

The ascorbic acid in this study at doses of 300mg/kg and 400mg/kg improved significantly the electrolytes namely the magnesium, sodium and chloride in the groups administered with higher doses. This due fact that ascorbic acid had been previously proven to have dehydration effect and this could be linked with improvement of electrolytes and homeostasis. The anti-oxidative property of ascorbic acid could also be involved in the protection of cell membrane and prevents compromise ionic exchange. This could also be having effect on osmolality and electrolytes regulation during stress. This is in agreement with previous study in which magnesium essentially is involved in ATPase regulation in glycolysis and metabolism (15). The ascorbic acid could improve the concentration of magnesium , this may be due to the fact that the supplemented ascorbic acid would sustain cellular antioxidative system to prevent the excess utilization and cell damage. This is the most likely reason why the magnesium in groups administered with high dose was higher. In the same vein, the supplementation of ascorbic acid would prevent dehydration in the animals. Previous observation in a research also supports the influence of ascorbic acid on body fluid and electrolytes (15). This would help in the regulation of metabolism and would prevent sodium and chloride ions loss as a result of stress. Previous literature had it that stress is accompanied with ions deficit (21). Ascorbic acid might also aid osmo-regulation and homeostasis to counteract the loss of sodium and chloride ions.

Ascorbic acid tends to increase the endogenously synthesized ascorbate concentration that would help to overwhelm the simulated reactive oxygen species radicals by mopping the radicals. This would help in depleting other endogenous antioxidant among which is GST. This may be the reason that administered doses of 300mg/kg and 400mg/kg had higher GST levels than other groups. However, in spite the antioxidative role of ascorbic acid the MDA, levels were higher in the treatment groups. This indicates that although ascorbic acid ameliorated stress, the long term transportation stress might have simulated much oxygen free radicals beyond the bearable level of the ascorbic acid administered. Therefore larger doses might be recommended as a probable solution to reverse higher level of the MDA in the treatment groups. The higher doses in this study did not improve the high level of MDA. Previous work (16) had documented the increase in the level of MDA and AST in stress in rats and this suggest that the level of MDA is dependent on the extent of stress to which the animals were subjected

Tables 5 and 6 shows the haematological effect of different doses of ascorbic acid in bucks exposed to long term stress. There was a significant ($P<0.05$) increase in red blood cells; the leucocytes and lymphocytes counts in all groups treated with ascorbic acid. The neutrophils-lymphocytes ratio was significantly ($P<0.05$) lower in the groups treated with higher doses of ascorbic acid (Table 6). The monocytes were significantly ($P<0.05$) increased in the group treated with 200mg/kg of ascorbic acid with a value of 1.11±0.64% when compared with 0.43±0.86% for the control.

Ascorbic acid increased significantly red blood cell count higher doses of 300mg/kg and 400mg/kg. It could be suggested that the cytoprotective effect of ascorbic acid helped in the maintenance of the cell membrane. Ascorbic acid decreased the tendency to osmotic fragility due to its free radical mopping effect and high cytoprotective property. This is the most likely reason for the changes in red blood cell count. The ascorbic acid might also have improved the metabolism which would have enhanced erythropoetic activity in the liver and other centres. There were changes in the percentages of the neutrophils, monocytes and decrease in the neutrophils-lymphocytes ratio in higher doses than lower doses.The changes were due to the effect of ascorbic acid in reducing the level of cortisol and adrenaline. Some previous workers also reported that ascorbic acid also has immunostimulating effect and this could also influence the cell-mediated immunity and haemogram in this study was in agreement with the work (23). This study further revealed that the immunomodulatory effect of ascorbic acid was dose-dependent with the higher doses having significantly higher values than the control. This study supports the fact that ascorbic acid alleviates stress by also improving the white blood cell count. Ascorbic acid at 400mg/kg and 300mg/kg elicited better immunomodulatory effects than lower dose used. It could be explained further by the fact that ascorbic acid scavenges the reactive oxygen radicals and this would protect the membrane of individual cells in the circulation and protect intravascular haemolysis due to stress which could cause destruction of blood cells .The findings of this study is in agreement with the previous findings stated (24), which found that ascorbic acid administration improved various haematological indices in goats.

Amongst the erythrocytic indices that were significantly increased by the ascorbic acid at 300mg/kg was the erythrocyte sedimentation rate (ESR), the reason for this might be the antioxidative property of the vitamin C. Treatment with ascorbic acid improved the ESR in the low density stocking (standard stocking). Ascorbic acid helped in the maintenance of the erythrocytes cell membrane integrity by stabilization of the phospholipid bilayer, thus reduces erythrocyte osmotic fragility and prevented haemolysis. In the same vein, the haemoglobin concentration was maintained within physiologic concentration with no significant difference between groups of ascorbic acid treatments. This infers that various experimental doses in this study played an important role in maintenance of erythrocytes parameters as reflected on the mean corpuscular haemoglobin and mean corpuscular volume. These findings are in agreement to the findings of previous studies conducted on ascorbic acid and erythrocytes and other haematological parameters at stress of transportation as recently observed in previous research (18) in pigs and (16) in sheep.

Conclusions and Applications

1. Ascorbic acid influenced the some vital physiological and biochemical parameters in long term transportation stress.
2. The higher doses improved the adaptation ability of the animals during the general adaptation syndrome of stress as during transportation of animals for a long time.
3. High ascorbic acid doses such as 300mg/kg and 400 mg/kg could be used to improve welfare of long road transportation stress of food animals such as goats.
4. The higher doses could be used to improve animal transportation when transporting animals from the Northern part of the country to the South during purchase for breeding and slaughter.
5. The supplementation of ascorbic acid is relatively safe and does not have residual effect thus, animals could be slaughtered without the marketers or butchers ensuring any withdrawal period.
6. The professionals in the livestock industry could also suggest to policy makers to the usage of this supplement to improve animal welfare.

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